Sensory Defects of Virgin Olive Oil from a Microbiological Perspective

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Virgin olive oil is the juice from the olive drupe, a produce that usually features high quality. However, virgin olive oils produced in different regions of the world suffer from defects, most of them of sensory nature. The largest amount of olive oil in which some organoleptic defect is present matches to the Virgin Olive Oil (VOO) class. The causes leading to sensory defects in virgin olive oil, on the different steps of the elaboration, are not well-known sometimes. Important is to note that current legislation allow the classification with sensory defects as VOO and Lampante Olive Oil (LOO). Volatile compounds are important in the sensory perception of the olive oils, for both the positive attributes and the defects. Knowledge on the biogenesis of the volatile compounds involved in the virgin olive oil defects and in its sensory characterisation, is still scarce. There are different causes of defects, depending on the physical, chemical or microbial degradation affecting the product. These join to the positive attributes, leading to an infinite diversity of sensory 'fingerprints'. The microbes cause the most of the defects sensory analysed in olive oils. Despite some interesting studies reported on the biogenesis of the compounds responsible of such defects, microbiological research within the olive oil production, including post-harvest life of olives, is scarce. We should highlight there is a long way to avoid the generation of sensory defects in olive oils within the production process itself and storage. This paper tries to identify significant shortfalls on the knowledge of the causes and circumstances for inducting sensory defects in olive oils, and defining the major aspects which are sufficiently known.

Keywords: fermentation, microbes, olive oil, quality, sensory defects.

Introduction

The mechanical procedures for elaborating oil from olives are different from those usual for preparing natural fruit juices. In fact the chemical constitution of these latter is mostly the watery solution of sugars, organic acids and pigments, whereas olive oil is mainly lipid. Overcoming these differences, the nature of olive oil is also the natural juice of the fruit's mesocarp, different to that of seed oils (Salas, Harwood & Martínez, 2013). Virgin olive oil is obtained through physical methods where the rules prohibit the use of solvents (International Olive Council, 2009), while it is the major method used for oilseed extraction (Rosenthal, Pyle & Niranjan, 1996). The olive oil which fulfils the conditions for direct marketing is classified as Virgin Olive Oil (VOO) or Extra Virgin Olive Oil (EVOO). Such olive oils must be of high or acceptable quality, both on their sensory and physic-chemical characteristics.

Olive oil is composed mainly of a saponifiable fraction accounting for 98.5-99.5% weight. This is formed by a lipid matrix of triglycerides, diglycerides and monoglycerides, these last two with about only 1.5% only of total fatty acids (Civantos, 1999a). Olive oil fatty acid composition is characterized by oleic acid as major part, providing a high monounsaturated-to-polyunsaturated fatty acid ratio, and it's also the major cause for the oil oxidative stability (Velasco & Dobarganes, 2002). Second, it contains a pool of minor but important compounds among lipids, such as phytosterols, waxes, carotenoids, tocopheroles and other as pentacyclic triterpenes. Squalene, an aliphatic hydrocarbon, is also in relatively high quantities within the minor component fraction, as well as compounds quite soluble in water like polyphenols, which stand out by its powerful antioxidant activity. Chlorophylls, aliphatic alcohols and terpenic alcohols are also present (Civantos, 1999a). We must remember that olive oil is not free from moisture, which can be up to 0.1%, playing an important role in developing microbial changes.

The current standards fixed by the International Olive Council differ slightly from that of the European Union (EU), the largest olive oil producer in the world, accounting for more than 80% (International Olive Council, 2015). In the EU (European Comission, 1991) the Standard needs for EVOO the presence of the fruity attribute and the complete absence of sensory defects, and other physic-chemical characteristics such a free acidity below 0.8%. In contrast, the VOO admits the presence of sensory defects below 3.5 on a scale of 10, and 2 % maximum acidity.

Those olive oils in which the sensory analysis decides the absence of fruity or/and the presence of sensory defects whose intensities exceed the value 3.5, classifies as Lampante Olive Oil (LOO). Thus, they must undergo refining, getting Refined Olive Oil (ROO). These olive oils are not edible, but only after mixing them with EVOO or VOO and classifies us Olive Oil (OO). OO may be manufactured as specific mixtures or 'coupages', which manufacturers produce according to the market.

EVOO is the most reputable olive oil. There is little information on the world production of the different categories of olive oils, especially in the case of LOO. Spain, the most important olive oil producer in the world, yields around 45% according the information available from the International Olive Council (2015). For this, we will use the production of Spanish olive oil as an example. The quantities of LOO and VOO vary over different seasons, as shown in Table 1. The sum of both classes varies in Spain from 45% to 55% of the total olive oil, the same in which sensory defects are present in greater or lesser extent depending on the produce. The data are elaborated from the Statistical Yearbook of the Spanish Ministry of Agriculture during the period 1980-2009 (Ministry of Agriculture, 1980-2009). In 2008 LOO represented 12% of the total Spanish olive oil, while in 2011 it was the significant amount of 23.5%. Nevertheless, the sum VOO+LOO was 55% of the total production in both years.

Table 1

We must highlight that LOO represents cases of very poor quality. The absence of the fruity attribute, the marked sensory defects, and the acidity above 3.3% are overall signs of great fruit or produce damage at any point in the olive oil elaboration. Nevertheless the largest amount of olive oil with some degree of sensory defect, until 3.5 of maximum intensity, matches to the VOO class. For the Spanish production, VOO accounts a yearly average around 40% of the total (Table 1).

The current legislation defines the classification and determines the quality of all olive oils commercially available. A part of this shows some intensity of sensory defects, as for VOO. Trading LOO occurs only within the industry, and its intensity of defects is higher than 3.5. The quantities of olive oil of different classes do not belong to the realms of Science but to Statistics. They provide a message of extraordinary importance: an outstanding percentage of the olive oil production has a certain intensity of defects, most of them of sensory nature. This is well known by experts in the olive oil industry, and we believe this data justifies the present work. The implicit message goes further if we try to answer the following question in detail: What are the causes and circumstances leading to sensory defects in olive oil? The answer is sometimes only loosely known. The purpose of this paper is to help defining what are known enough, defining aspects suffering from significant shortfalls, on the knowledge about the causes and events inducting sensory defects in olive oils.

Sensory quality of olive oil

Olive oil sensory quality is described mainly according to volatile compounds, whose detailed description was reported recently (Aparicio, Morales & García 2012). Around one hundred and eighty compounds, were found to be responsible of virgin olive oil aroma (Olías, Del Barrio & Gutiérrez, 1977; Del Barrio, Gutiérrrez & Gutiérrez, 1981; Morales, Aparicio & Ríos, 1994; Angerosa, 2002; Procida, Giomo, Cichelli & Conte, 2005; Morales, Luna & Aparicio, 2005). Thereby, specific relations between volatile compounds and sensory attributes and defects of olive oils have been established. Volatiles can be formed and then released from the olive oil matrix, but they can be native components too remaining within the product (Schreider, 1984), and participating not only in olive oil odour but also in its flavour.

There is also a part on olive oil flavour which is not due to volatile compounds. This is the case of the ‘bitter’ and ‘pungent’ attributes, two components that can clearly emphasise the flavour of many types olive oils which contribute positively to the sensory quality (International Olive Council, 2013a). Oleuropeine and its aglycon form are especially responsible, among the phenolic compounds, for the bitterness of virgin olive oils (Caponio, Gomes, & Pasqualone, 2001). While, oleacein and oleocanthal are responsible for the burning pungent sensation of certain olive oils (Andrewes, Busch, De Joode, Groenewegen, & Alexandre, 2003). These attributes, however, are not of much consideration in the commercial standard, since they are not decisive in olive oil classification. Many other attributes are named by their likeness to different flavours in which ingredients other than volatile compounds are present. Such are the cases of *fresh herb*, *apple, white fruit*, *green fruit*, *nutty*, *almond*, or *tomato*.

The knowledge on the biogenesis of the volatile compounds that participate in olive oil sensory quality is little, despite the existence of important research (Olías, Del Barrio, Gutiérrez, 1977; Olías, Pérez, Ríos, & Sanz, 1993; Luaces, Pérez, & Sanz, 2003; Angerosa, Servili, Selvaggini, Taticchi, Esposto, & Montedoro, 2004; Sánchez-Ortiz, Pérez, & Sanz, 2007; Sánchez-Ortiz, Pérez, & Sanz, 2013; Reboredo, González, Cancho, & Simal, 2013). Green odour biogenesis of some virgin olive oils has been studied, leading to a possible pathway for the C6 polar volatile compounds. According to this route, a lyase would cleave the 13-hydroperoxides (C13-OOH) of linoleic and linolenic acids to form the volatile aldehydes hexanal and cis-3-hexenal, respectively. This pathway involves other enzymes such as acyl hydrolase, lipoxygenase, hydroperoxido lyase, alcohol dehydrogenase and alcohol acetyl transferase (Olías, Pérez, Ríos & Sanz, 1993; Kalua, Allen, Bedgood, Bishop, Prenzler & Robards, 2007).

Short chain alcohols present in olive oil aroma are responsible for its characteristic and appreciated ‘green notes’. Salas & Sánchez (1998) suggested that these short chain alcohols are formed from the aldehydes by a NADP-dependent alcohol dehydrogenase (ADH) present in the mesocarp of developing olives. This points the relation between the alcohol-dehydrogenases of olive fruit and the volatile’s biogenesis.

A study on the lipoxygenase activity within the olive fruit development have revealed its significance in the biosynthesis of virgin olive oil aroma. It was reported that olives showed the highest lipoxygenase activity about 15 weeks after anthesis, with a steady fall during the growth and ripening periods (Salas, Williams, Harwood & Sánchez, 1999). From here, those authors proposed that olive lipoxygenase engages in the biogenesis of six-carbon volatile aldehydes. The same authors highlight that six-carbon volatile aldehydes are major constituents of the aroma of virgin olive oil during the extraction. Modulating the aroma’s biogenesis produced from the alcohol- dehydrogenase of *Olea europaea* in the fruit is other interesting aspect that has been described (Iaria et al. 2012). Characterising the lipoxygenases has been further studied in some olive cultivars, showing again their role in the synthesis of volatile compounds (Ridolfi, Terenziani, Patumi & Fontanazza, 2002). The enzyme alcohol acyltransferase from olive fruit has been characterised too (Salas, 2004). Sánchez-Ortiz, Pérez & Sanz (2007) reported cultivar differences on non-esterified polyunsaturated fatty acids as a limiting factor for the biogenesis of virgin olive oil aroma.

Also, Salas & Sánchez (1999) identified the inactivating of hydroperoxide-lyase by malaxation at temperature high as cause of drop of the flavour of virgin olive oil. The role of the olive seed in the biogenesis of virgin olive oil aroma is an interesting issue. Olive seeds would afford an alcohol acyltransferase that might be unspecific for substrate, producing any kind of esters (Luaces, Pérez & Sanz, 2003). Further, the role of the pulp and seed in the biogenesis of extra EVOO aroma through the lipoxygenase (LOX) pathway has been studied in four cultivars (Arbequina, Picual, Local and Manzanilla de Sevilla). C6 and C5 volatile compounds were revealed responsible for EVOO aroma, which were produced by endogenous enzymes in both parts of olive fruits. It was shown that these compounds have mainly their biogenesis in the pulp (80–90%) instead of in the seed (20–10%), independently of the cultivar considered (Reboredo, González, Cancho & Simal, 2013).

The second essential part of the sensory quality of olive oil is the sensory defects. The different types of sensory defects of olive oils are in the quality standards of both the EU and the International Olive Council. There are different causes of defects, depending on the physical, chemical or microbial degradation of the product. Such degradations may occur concomitantly, inducing a set of chemical and sensory negative attributes, which together with positive ones leads to an infinite diversity of sensory fingerprints of the virgin olive oils.

The main defects that can be detected in certain olive oils, from the highest to the lowest frequency, are the following. *Fusty* flavour develops when olives are stored in piles, favoring anaerobic fermentation. *Mouldy* is characteristic of olive oil got from wet olives, stored for days, in which fungi and yeast have developed. *Muddy sediment* defects appear when the olive oil has been in contact with the sediment in vats or tanks. *Winey-vinegary* is characteristic of certain olive oils with wine or vinegar flavour, due to the fermentation responsible for producing acetic acid, ethyl acetate and/or ethanol. *Rancid* flavour is characteristic of olive oil that has undergone oxidation, with full correlation between the intensity of the defect and the degree of oxidation. *Metallic* comes from prolonged contact with metallic surfaces during crushing, mixing, pressing or storing. *Heated* or *burnt* attributes are usually due to excessive and/or prolonged heating during processing, especially in the thermo-mixing of the dough. *Alpechin* is the olive oil flavour received by prolonged contact with vegetable water. Other defects are *hay-wood*, due to dry olives and *brine*, typical of olive oils extracted from olives with brine conservation. *Earth*, is typical of olive oils got from fruit that have been in contact with the earth or mud or improperly washed. There are also defects that occurs rarely, as the *lubricant* flavour, reminiscent of diesel, grease or mineral oil and whose origin would likely be the contact with lubricants extraction machinery.

One relevant facet on olive oil sensory defects concerns to fatty acid alkyl esters (FAAEs). FAAEs originate by esterification of free fatty acids with short chain (from one to four carbon-atom) alcohols, mainly methanol and ethanol yielding methyl and ethyl esters, respectively (Gómez-Coca, Moreda, & Pérez-Camino, 2012). When FAAEs are present at a certain concentration, the fermentative change becomes clear (Pérez-Camino, Moreda, Mateos & Cert, 2002). From here, a relationship between FAAEs concentration of olive oils and its fermentative sensory defects have been established, as well as with their sensory classification (Gómez-Coca, Moreda, & Pérez-Camino, 2012). The esterification needs the presence of short chain alcohols produced by fermentation and olive oil free fatty acids, according to the same authors.

The adoption of the FAAEs as quality parameter to classify olive oil either as virgin or extra virgin took place some years ago (International Olive Council, 2010; European Commission, 2011). However, today the relationship between FAAEs and some sensory defects, and the convenience of the current limits, are questioned. Therefore, in 2013 new official requirements were established, the FAAEs maximum allowed limit reduced to 40 mg.kg-1. Additionally, decreasing such threshold 5 mg.kg^{-1} by year within the two following, was scheduled (International Olive Council, 2013b; European Commission, 2013). Rapid methods for predicting sensory defects have been reported (Sinelli, Cerretani, Egidio, Bendini, & Casiraghi, 2010; Lerma-García, Cerretani, Cevoli, Simó-Alfonso, Bendini, & Toschi, 2010; Cayuela, Peña, & García, 2013). These technologies, different to the insight above, would be useful to prevent mixing batches of defective virgin olive oils with those without specific quality problems.

The volatiles involved in the major defects of virgin olive oil have been described by several authors (Del Barrio, Gutiérrez & Gutiérrez, 1981; Procida, Giomo, Cichelli & Conte, 2005; Morales, Luna & Aparicio, 2005; Aparicio, Morales & García 2012). Most of them are due to microbes. The driving force in the research above is revealing the enzymes forming the characteristic volatiles of defective olive oils (Angerosa, Lanza & Marsilio, 1996; Angerosa, Lanza, D'Alessandro, Marsilio, & Cumitini, 1999; Olías, Pérez, Ríos, & Sanz, 1993;Sánchez-Ortiz, Pérez, & Sanz, 2007). It is important considering whether they come from the olive or they are produced by microorganisms, which generally has not so far been the main objective in these studies.

Each of *muddy*, *mouldy*, *fusty* and *vinegary* flavours involves many volatile compounds. These are different depending on the produce, according to several authors (Angerosa, Servili, Selvaggini, Taticchi, Esposto, & Montedoro, 2004; Aparicio, Morales, & García-González, 2012; Gutiérrez, Dobarganes, Gutiérrez, & Olías, 1981; Morales, Luna, & Aparicio, 2005; Olías, Del Barrio, & Gutiérrez, 1977). Some of them highlight by their quantitative importance, according a summary (Table 2) of data reported by Procida, Giomo, Cichelli & Conte (2005), referencing to several studies which have been previously reported.

The *vinegary* pattern is a special case since acetic acid and ethylacetate accounts much than 70%. Ethanol and methanol, respectively responsible for some 16% and 5% of the total composition of volatile in this olive oil pattern, are also important. The fusty pattern includes mainly ethanol (38%), octane (14%), ethylacetate (12%) and methanol (11%). In the *muddy* pattern highlights ethanol (30%), and in proportions between 10% and 5% butylformate, ethylacetate, 2-butanol, 1-propanol and ethylpropionate (Procida, Giomo, Cichelli & Conte, 2005).

The volatile compounds total in the *mouldy* pattern is less than in the remaining major defects, according the same data. In this, ethanol and hexanal, represents about 20% of the total, methanol around 12% and in proportions between 4 and 10% propanal, pentanal and propylpropionate. Moreover, in this pattern none volatile represents much more than 20% of total among 38 detected compounds. Important is highlighting that in many cases, compounds of very low concentration are major responsible for the defect, as can be in the *mouldy* flavour. Thus, compounds with high odour threshold values have less sensory significance than those with low odour thresholds (Luna, Morales & Aparicio, 2006). The odour activity values (OAVs) of volatile compounds, defined as the ratio of the concentration to the odour threshold, is used to define their sensory significance (Rothe & Thomas, 1963; Aparicio & Morales, 1998; Buettner & Schieberle, 2000). The off-flavour compounds are potentially toxic and have low odour thresholds (Angerosa, 2000). However, we have to underline that beyond its organoleptic significance, the data included in Table 2 signal the origin of the sensory defects that is more important for our current purpose.

In this sense, we must highlight the quantitative importance of ethanol in *muddy*, *mouldy*, *fusty* and *vinegary* flavours. Methanol as well is present in the last three, and it is noticeable that its odour threshold in olive oil has not been reported. Both volatile reveals a remarkable influence of the alcoholic fermentations in the sensory defects most normal in the virgin olive oils. Worth to note this can have a direct relation with yeast fermentations, produced at any point of the manufacturing or storage.

Table 2

Sensory defects from microbes

One of the facets of olive oils' sensory defects known worst is what are the circumstances leading to producing undesirable flavours from microbial causes, within the virgin olive oil extraction. The *fusty*, *mouldy*, *winey-vinegary*, and *muddy* sediment are in fact caused by microbes. Moreover the basic defects mentioned above are recognised as families of defects, which show some overlap. The storage of olives in piles induces fermentation which can carry into dramatic sensory defects. The biogenesis of *fusty* defect has been studied by chemical and microbial analysis in olives stored in piles and their resulting olive oils (Gutiérrez, R., Dobarganes, M.C., Gutiérrez, F., & Olías, J.M. (1981). The quali-quantitative composition of the olive oil volatile fraction was reported as good marker of some metabolites produced by microorganisms involved during the fruit storage (Angerosa, Lanza & Marsilio, 1996). The above authors indicated that some volatile compounds, such as 2- and 3- methyl butan-1-al, their corresponding alcohols and propionic acid, 2-methyl propionic acid and 3-methyl butanoic acid, are produced. It was observed in the same study a dramatic development of *Clostridium sp*. and in a lower proportion, of *Pseudomonas sp*., which produce branched aldehydes, branched alcohols and their corresponding acids, surpassing in a few days the threshold levels for sensing *fusty* defect (Angerosa, Lanza & Marsilio, 1996). The possible presence of *Acetobacter* is responsible, according the same authors, for the *vinegary* defect because of producing acetic acid. Besides, softening fruits during the storage has been attributed by the same authors to moulds and other microorganisms in rapid growth belonging to *Enterobacter sp.*

Angerosa, Lanza, D'Alessandro, Marsilio, & Cumitini (1999) studied the modifications of volatiles in virgin olive oils by *Aspergillus parasiticus* and *Penicillium nalgioviense*, responsible for *musty* defect. For this purpose, the above authors compared the volatiles of olive oil samples from fresh fruits with those oils extracted from olives inoculated with mould cultures. Their results point out that trans-2-hexenal disappears, as well as the presence of 3-octanol and 1-octen-3-ol, typical metabolites of the inoculated cultures.

Meanwhile, yeasts can produce great amounts of ethanol and ethyl acetate, especially with temperature warm, inducing the *winey* defect (Angerosa et al., 2004). Interesting results were reported by Fakas et al. (2010), which focused on characterising olive fruit microflora and its influence on olive oil volatile compound biogenesis. Olive fruits of the Greek variety 'Amfissis' were stored under industrial conditions, its microflora isolated and identified. The above authors selected moulds, and screened it for production of lipase and lipoxygenase. These last are two enzymes known to act during the biogenesis of olive oil aroma through the lipoxygenase pathway. A *Penicillium* strain was identified as the most potent enzyme producer, and its production of lipase and lipoxygenase quantified. The authors reported that olive microflora can contribute to the biogenesis of olive oil volatile compounds. Thus, some of its members could potentially be used to increase the olive oil aroma.

For understanding why the microorganisms are involved in the olive mill scene, we should point out that a few decades ago it was still frequent storing the olives in large lashings. The cause was they were waiting the moment of grinding, because of the mills' limits in particular areas (Gutiérrez, R., Dobarganes, M.C., Gutiérrez, F., & Olías, J.M., 1981). Currently the waiting time depends on the extraction method, since the milling rate differs markedly in the methods known as *traditional*, *two-phases*, and *three phases* (Civantos, 1999b). When the height of the olive in the pile exceeds certain limits, the lower layers of the product suffer squashing and even crushing. Then, this generates tissue damage, rupture of the fruit's pericarp and liquid leak, favouring the conditions for acting various microorganisms (Gutiérrez, R., Dobarganes, M.C., Gutiérrez, F., & Olías, J.M., 1981). The circumstances above bound to the presence of the fruit mechanical damage during harvest, formerly performed mostly by shaking the olive trees with chestnut rods, a method increasingly fewer used. The main causes of mechanical damage include unsuitable harvest methods, damage in transport, damage due to singularities in the travel from the receiving hopper to the olive mill, and olive height excess in the piles. However, the compounds produced by microorganisms are not only volatiles, nor only present in the olive piles.

Major cause of microbial spoilage, with fruit’s mechanical damage, is the degree of cleaning and the presence of moisture. Contamination with ground or mud is frequent (Alba, 2013). Also olives are normally wet, since the harvest time coincides with the fall, a rainy season in Europe. Therefore, microbes growth in each batch of olives will largely depend on its conditions specific on the above factors.

Besides the microbiological contamination after harvesting as well the mechanical damage suffered by the olives before milling, one must see the whole line of olive oil production. After grinding, the master miller, cares on preserving the cleanliness of the different mechanical in contact with the olive paste or the olive oil.

One wonders to what extent upgrading the entire production, which is a proven fact from the olive tree flowering to olive oil packaging, has led to improvement of the quality of olive oil. According to data from the Statistical Yearbook (Ministry of Agriculture, 1980-2000) shown in Table 1, the percentage of EVOO from 1980-2000 was relatively stable around 55% of the total. In the middle of the last decade this figure was somehow lower. There are several possible considerations about standards changes, which affect the olive oil quality in the statistics. However, it is objective to say there is a long way to avoid the generation of sensory defects in olive oils within the production itself. We believe that progress in this direction should continue.

Critical points in elaborating olive oil

It is well known that olive oil free of foreign substances is resistant to microbial spoilage, since it contains polyphenolic compounds with high antimicrobial activity (Cicerale, Lucas & Keast, 2012). Microbial growth is favoured by the presence of other substances in olive oil. These are mainly solid fruit rests and watery fraction, as well any possible contamination from soil that has been able to pass after fruit washing before milling. Then, the chemical changes provide substrate for the microorganism development. These conditions can occur in the following critical points.

The mill

In the olive mill itself, even after cleaning, traces of moisture, mesocarp, endocarp and seeds may remain. Although the volume inside this is small, this fact may be more common than people thinks, when the cleaning is weak. This waste material affects the next olive batch. An ‘old’ olive paste fraction altered by microorganisms will join the fresh product, with the consequent risk of defect in the final virgin olive oil (Alba, 2013). Characterising the microorganisms that act at this point and the specific changes they can induce, remains unknown to the best of our knowledge.

The beater

When the olive paste contains an inoculum microbial, the paste beating is providing the conditions for microbes growth and the related disturbances. Usually the beating temperature recommended is below 30 °C, although depending on the characteristics of the dough this feature can be at higher limits (Civantos, 1999a). Sometimes an excessive temperature of the olive paste beating leads directly to the defect known as burnt or heated, as already mentioned. An olive mass at 30 °C carrying a specific microbial inoculum can remember to wheat flour’s dough voluntarily inoculated with yeast, which kneads in the bread machine. In this case, after 30 minutes fermentation time, the yeast grows and is perceptible. One may think that something similar can happen at a different scale in some fractions of the olive paste. A large gap exists between these two cases, especially for the important capacity of the olive phenolic compounds to inhibit large number of microbial species (Soler-Rivas, Espin & Wichers, 2000). The inoculum necessary can easily come from the mill machine because of the difficulty in cleaning the small areas in the beater. This possible microbial when beating the olive paste remains not described and not quantified, but it is reasonable to think it happens.

Trays, sieves and decanter

In this case we will focus on the two-phase system, used mainly. Once completed the beaten of olive paste, a pump pushes it into the centrifugation machinery. The centrifuge must be susceptible of a proper cleaning. However, it is not inconceivable there might be pipe's singularities, difficult to cleaning and potential hot spots prone to microbial growth.

After separation of the olive oil from the solid debris and the watery phase within the decanter, the produce passes a series of sieves. Each of these mechanics, the decanter in particular, is susceptible to cleaning defects (Alba, 2013). Worthy is to consider possibility of new machinery designs for an easier cleaning, which could provide valuable improvement. Therefore, foam's appearance in a quantity that depends on the cleanliness is not uncommon, providing a substratum susceptible to alcoholic fermentation. In these circumstances, alcoholic fermentations can produce certain quantities of ethanol or methanol. Thereby providing the necessary substrates to form alkyl esters and for incorporating sensory defects to olive oil (Gómez-Coca, Moreda, & Pérez-Camino, 2012). As in the precedent mechanic spots, the description and characterisation of the microorganisms acting at these points, and its specific relation with the different olive oil sensory defects remains unreported.

Tanks

Ideally, the tanks used for olive oil storage from different classes should be free of any material other than olive oil. This means the tanks must be free of contamination from any water fraction or any other matter. However, the olive oil normally has some moisture. Moreover it is usual the condensation of water from the air contained in the tank, due to lowering the environmental temperature, which results in the existence of a watery fraction. This is the ingredient necessary for lipid hydrolysis and free fatty acid (FFA) generation by chemical or enzymatic actions (Alba et al., 1996). These FFA released can crumble into shorter chain fatty acids, causing bad odours and flavours (Civantos, 1999a). This is the reason it is advisable to replace the air in the empty part of olive oil deposits with nitrogen (Leone, Romaniello, & Tamborrino, 2013).

The olive mill's microbiology

The olive pomace and the olive cake has been the subject of intense microbiological research (Maestro, Borja, Martín, Fiestas & Alba, 1991; Borja, Martín, Alonso, García & Banks, 1995; Millán et al., 2000; Borja, Rincon & Raposo, 2006, among many others), thus the existing knowledge is extensive. By contrast, microbiological research within olive oil elaboration, including post-harvest life of olives, is little.

Describing the microorganisms involved in inducing olive oil sensory defects within the olive mill, can be useful. Especially about their taxonomic diversity, their specific growth conditions and the sensory defect related. Identifying species affecting each substrate and defining its biology and its final products may be important, as well as the possible specific relation microorganism-defect. It is desirable to increase the awareness on the need of protocols from the perspective of the microbiological hygiene. Ensuring the cleanliness of each mechanic unit in the extraction would allow improving the quality of the olive oil.

Can assume that most micro-flora in the mechanic units of the olive mill come from the olive tree. There may be another part from the agro-ecosystem in which the industry is established, soil microorganisms mainly. Olive paste is a medium in which a high number of fungal species is present. An example of this is a recent study in which fifty-three fungi strains were isolated in olive products (Alves, Romo-Sánchez, Úbeda-Iranzo & Briones-Pérez, 2012). The authors found ten different species in the olive paste, followed by the olive fruit and the olive pomace, with five species each. Among all those species, six were typically of olive paste (*Penicillium chrysogenum*, *Rhizopus oryzae*, *Lichtheimia corymbifera*, *Penicillium crustosum*, *Mucor circinelloides* and *Aspergillus niger*), whereas *Mucor fragilis* was found only in fresh olives and *Rhizomucor variabilis* var. regularior only in olive pomace. The main objective of such a study was identifying enzymes with potential biotechnological utility, thus no mention olive oil quality.

Olives are probably the main vehicle arrival of microbial population to the mill. Yeasts are especially abundant in the olives, with Pseudomonads, lactic acid bacteria and Enterobacteriaceae in a lower account, according to data reported by Tassou, Panagou & Nychas (2010). As well, the microflora of olive fruits of the Greek variety 'Amfissis' were studied (Fakas et al., 2010), and a wide number of microbial species reported, as *Fusarium* spp, *Penicillium* spp, *Aspergillus* sp. *Rhizopus nigricans*, *Paecilomyces* sp. and *Verticillium* sp., as well yeasts belonging to the genera of *Candida*, *Cryptococcus*, *Pichia*, *Rhodotorula*, *Debaryomyces*, *Saccharomycodes* and *Wickerhamiella*. Gram positive bacilli and cocci were also identified, as that from the genera *Actinomyces*, *Corynebacterium*, *Listeria*, *Pseudomonas* and *Vibrio*, although only their lipase and lipoxygenase activities were investigated. We must highlight that these data agrees with the importance of ethanol and methanol in *muddy*, *mouldy*, *fusty* and *vinegary* flavours, before referred (Table 2). It is logical to think there are differences in the microbial population from the olives surface among different geographical origins and varieties. Could be this fact related to some of the sensory characteristics of virgin olive oils?

Olive oil contains solid particles and micro-drops of vegetation water which settle during the produce's storage. Noteworthy is the unfiltered virgin olive oil is found in the market. In fact, avoiding olive oil filtration is reported as desirable for extending olive oil's shelf life (Frega, Mozzona, & Lercker, 1999). The reason given is the dispersed particles play a double stabilising effect on both oxidative and hydrolytic degradation. However, suspended matter in the olive oil or that deposited on the bottom of the tanks could contribute to fermentation, since the lack of filtering allows moisture to remains in the olive oil, thereby the microbial content can be higher. In fact, Ciafardini & Zullo (2002a) reported that together the suspended material in the olive oil, there are a rich microflora mainly composed of yeasts. Besides, the same authors shown the partial elimination of the sediments in extra virgin olive oil, carried out with cotton filtration by the bottling companies, allows a significant decrease of the number of micro-organisms.

Certain microbial species communicate certain desirable attributes to many foods such as dairy and meat products, bread, wine and other drinks, as well table olives. The question is, could certain virgin olive oils obtain some benefits from microorganisms the way other products do? This sets up a captivating field of research, and there are already some evidences. Some of the virgin olive oil yeast microflora is useful, as they improve the organoleptic characteristics of the olive oil during preservation (Zullo, Cioccia, & Ciafardini, 2010). The same authors point out that other yeasts are harmful, as they can damage the quality of the olive oil through the triglycerides hydrolysis. Yeasts in stored olive oil have been studied (Ciafardini & Zullo, 2002b), showing that oleuropein present in olive oil can be hydrolysed by β-glucosidase from the yeasts *Saccharomyces cerevisiae* and *Candida wickerhamii*. The authors also noted the absence of lipases in the mentioned yeasts, suggesting their positive contribution to olive oil sensory quality, without altering the triglycerides composition. In the other hand, the influence during olive oil storage on its sensory characteristics of some yeast strains belonging to *Candida spp* have been recently reported (Zullo, Cioccia, & Ciafardini, 2013). The authors above have shown that all the tested yeasts, used to inoculate EVOO, survived more than four months of storage. In the same work *muddy-sediment*, r*ancid* or both defects were found in olive oil samples treated with *C. adriatica*, *C. wickerhamii* and *C. diddensiae* specific strains. In the same study, olive oil samples treated with *C. diddensiae* strains were defect-free after four months of storage, and still categorized as extra virgin.

Therefore it seems obvious that a deeper understanding of the microbiological events which happen in the different steps of the olive oil mills, will be welcome. This would contribute to clarify the influence of microorganisms in shaping the enormous diversity of sensory 'fingerprints' of the virgin olive oils.

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Season	EVOO	%	VOO	%	LOO	%	VOO+LOO	%	Tota
1980-1985	254,522.33	55.0	191,679.83	41.0	16,284.33	4.0	207,964.17	45.0	462,48
1986-1990	295,103.20	52.4	230,416.00	41.0	37,832.20	7.0	268,248.20	47.6	563,35
1991-1995	310,501.80	58.8	201,188.00	38.0	16,117.80	3.0	217,305.80	41.2	527,80
1996-2000	456,242.00	52.9	345,280.25	40.0	61,482.50	7.0	406,762.75	47.1	863,00
2001-2005	562,518.60	50.3	419,702.80	38.0	136,287.40	12.0	555,990.20	49.7	1118,50
2006-2009	503,936.25	45.4	398,986.50	36.0	207,646.50	19.0	606,633.00	54.6	1110,56
Average		52.5		39.0		8.5		47.5	

Table 1. Virgin olive oil production in Spain (t). EVOO, Extra Virgin Olive Oil; VOO, Virgin Olive
Lampante Olive Oil. All the figures express in yearly averages. (Own data from: Ministry of Agricul
Government of Spain. Olive. In *Statistical Yearbook*, years 1980-2009 (Chapter 14). Accessed March
http://www.magrama.gob.es/es/estadistica/temas/publicaciones/anuario-de-estadistica

Compound	OT	Muddy	Mould	Fusty	Vinegary
Methanol	33.00[a]		11.9	11.1	4.7
Ethanol	30.00[b,c]	29.5	20.1	38.2	16.4
Propanal	0.01[d]		8.2		
1-Propanol	2.60[a]	5.1			
2-Butanol	0.15[c]	6.9			
Ethyl acetate	0.94[b]	8.4		11.6	73.1
Butyl formate	0.87[a]	10.2			
Pentanal	0.24[e]		4.2		
Ethylpropionate	0.10[b]	5.8			
Hexanal	0.08[b]		19.9		
Octane	0.94[b]		6.1	13.7	
Acetic acid	0.50[b]				

Table 2. Main volatile compounds (%) in olive oil defects patterns due to microbial alteration. Summarized from Procida, Giomo, Cichelli & Conte (2005). OT, odour threshold (mg/Kg). a) Odour thresholds not reported for olive oil, but measured by the triangle odour bag method (Nagata & Takeuchi, 1990). [b]Purcaro, Cordero, Liberto, Bicchi,